Notes on the Free-Living Nematodes.

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With 11 Text-figures.

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Introduction.

Orley divided the Nematoda into three groups, roughly corresponding to differences of habitat found in the phylum. (1) Nematozoa embracing all parasitic forms, (2) Rhabditiformæ which live free in "decomposing organic substances or in earth saturated with such substances"; and (3) Anguillulidæ, the rest of the free-living nematodes, found in soil or water. Such a classification, grounded on œcology, pays no attention to the facts of morphology, and is naturally out of place in zoological arrangement, which aims at expressing the relationship of animals by descent. The methods of life of an animal are, moreover, largely ruled by the mode of procuring nutriment which has been adopted. The first two groups of Orley are parasites and saprophytes respectively, but in the Anguillulidæ we have a heterogeneous collection of forms varying greatly in their habits of life. Little is known of their sources of nourishment save in the case of a very definite division (e.g. Tylenchus, Dorylaimus), which live on the juices of plants, and for that end are provided with a small protrusible spear for piercing tissues and suctorial pharynx for absorbing sap thus set free. The vast majority of this family, however, possess an unarmed buccal cavity; but in all the muscular pharynx is constantly at work, now dilated, now collapsed, constantly pumping fluid through the alimentary canal. There is no morphological distinction to be observed between such a free-living nematode as is found in the mud of a lake or amongst the algæ of the marine littoral and a Rhabditis or Diplogaster of the soil. But the latter class can be kept in a culture fluid which swarms with bacteria, in which individuals of the former class would speedily succumb. The tissues of a Rhabditis must be resistant to bacterial action and unharmed by the toxins which such organisms produce, and the worm is, in fact, capable of building up protoplasm from the bacteria themselves or from the products of their action. These are the most prominent physiological characteristics of the soil nematodes, Orley's Rhabditiformæ, and account for the peculiarities of their distribution, for they are apparently absent from dry soils and those with a small admixture of organic matter, and even in soils rich in humus are only detected in quantity by allowing some animal or vegetable substance to putrefy on the sample. Sufficient attention has not been paid to the part which nematodes play in the economy of the soil,[1] but an investigation of this problem may well reveal results of as great interest as those which have been put on record by Maupas, working on the sexual organisation. In the present paper it is proposed to confine attention to the reproductive phenomena in certain hermaphrodite species, but it is hoped in a subsequent research to return to the nutrition and distribution of the class.

Cultures of free-living nematodes in connection with this work were first started at the Stazione Zoologica, Naples, in 1906, and continued at intervals in the next two years at the Zoological Laboratory, Cambridge, using for the most part Diplogaster linstowi. In 1909 I spent July to September at the Sutton Broad Laboratory, Norfolk, and procured from the neighbourhood the two forms, Rhabditis gurneyi and Diplogaster maupasi, the study of which enables me to amplify in one or two particulars Maupas' account of the free-living hermaphrodite species of nematodes. I wish here to express my sense of the value of the opportunities for research afforded by the Sutton Broad Laboratory, and to thank Mr. Robert Gurney for his great kindness to me while working there.

Summary of Sexual Phenomena in the Hermaphrodite Species.

Guido Schneider, in his 'Monographie der Nematoden' (1866), first discovered and put beyond doubt the existence of self-fertilising hermaphrodite species of free-living nematodes.

[1] The importance of the protozoan fauna of soil has but recently been realised. Like that of the nematodes their nutrition is composed of bacteria, and the place they take as a limiting factor in the increase of nitrifying forms has the closest possible bearing on the fertility of the soils they inhabit. It is, however, probable that these protozoa are more widely distributed in soil and so exercise a more important influence. (See E. J. Russell and H. B. Hutchinson, 'Journ. Agric. Sci.,' vol. iii, 1909, "The Effect of Partial Sterilisation of Soil in the Production of Plant Food," especially p. 141.)

In 1900 Maupas,[1] in a brilliant paper, drew attention to many striking features in the reproductive phenomena of such species. A full description of all prior work relating to hermaphroditism in the Nematoda is given by Maupas, and I shall here content myself with a short resumé of his own results, which later will be quoted more in extenso in connection with my own observations.

The species of the free-living nematodes Rhabditis and Diplogaster fall into one or other of three categories:

(1) Bisexual species, in which male and female individuals are produced in equal numbers.

(2) Hermaphrodite species, in which, besides the self-fertilising protandrous hermaphrodites which form the great mass of the species, there are occasional male individuals, perfectly developed apparently, but taking no part in reproduction.

(3) Parthenogenetic species, in which males have not been found.

It is reasonably supposed that each hermaphrodite species is derived from a bisexual form by the development of spermatozoa in the ovary of the female individuals, which thus become self-fertilising. The males are now useless, and have even to a large extent lost their sexual instinct. Their number dwindles in most cases to an almost imperceptible figure, but final disappearance does not appear to be reached in any species, and this persistence of apparently useless forms is one of the most curious facts recorded in biology.

The hermaphrodite species appear even more numerous than the bisexual. There is, indeed, some evidence that the conversion of females to hermaphrodites in the bisexual species is a present-day process, furnished by the examples of partial hermaphroditism described by Maupas. An intermediate condition is shown in some hermaphrodite species by the occasional occurrence of pure females, or in the production of

[1] E. Maupas, "Modes et Formes de Reproduction des Nématodes," 'Arch. de Zool. Exp. et Gen.,' Sér. 3, t. 8, 1900, pp. 463–624, Pls. xvi–xxvi.

spermatozoa in one half of the genital gland only, the other producing eggs alone. Maupas emphasises the significant fact that these species with an incipient hermaphroditism yield the highest proportion of males he was able to chronicle. This conclusion that the more complete development of hermaphroditism and the suppression of the male sex necessarily proceed closely together is discussed further below.

It is also highly characteristic of the hermaphrodite species in general that the sperm each individual produces only suffices for the fertilisation of a limited number of eggs, so that the period of fertility is followed by one even more prolonged, during which unfertilised eggs are laid, which do not develop. Such a phenomenon marks the hermaphroditism of the free-living nematodes as a character comparatively recently acquired and as yet not shaped by natural selection in anything like its final form.

Finally, a most interesting result was obtained by experiments with hermaphrodites which had exhausted their stock of spermatozoa and supplemental males of the same species. In the rare occasions in which fecundation took place the eggs which were afterwards laid produced males and females in equal numbers.[1]

Systematic Part.

Diplogaster M. Schultze.

This genus includes representatives both from soil and fresh water. But while the former possess a weakly developed bursa, which indicates the relationship of the genus to Rhabditis, the latter are without this character, and this fact, according to Bütschli, affords a natural distinction between the classes.

[1] A preliminary note published in 1908 ("Sexual Phenomena in the Free-living Nematodes," F. A. Potts, 'Proc. Camb. Phil. Soc.,' vol. xiv, Pt. IV, pp. 373–5) gave a general confirmation to Maupas' results, founded on observations on Diplogaster linstowi which was kept in cultures for over a year and then died out.

The soil-nematodes belonging to this genus differ widely among themselves, particularly in respect of such definite characters as the number and arrangement of the papillæ on the tail of the male. The typical number is nine or ten pairs, but D. gracilis Bütschli and others have eight, and D. robustus Maupas, eleven. The arrangement of the papillæ is more variable than their number, but in a small group of species, with which I am more specially concerned here, the relative positions are fairly constant and characteristic.

The arrangement of the papillæ follows the scheme given below. The numbers correspond to those given in the various diagrams (see Text-fig. 4).

(1) A pair of papillæ opposite the anterior end of the copulatory spicules. D. robustus Maupas possesses an extra pair, situated far in front of the spicules. In D. maupasi sp. n., as a frequent variation one of this pair may have been shifted forward to a markedly pre-spicular position.

(2, 3) Two pairs of papillæ opposite the posterior end of the copulatory spicules.

In D. robustus Maupas shows three pairs in this position.

(4) One pair slightly post-spicular.

(5, 6) Two pairs, the anterior situated about half-way between the root of the tail and the anus, and the posterior at the root of the tail.

(7–9) Three small pairs at the root of the tail, more ventral than the last-named.

Since, then, there is so much similarity between the members of the group, the species are best distinguished by differences in size, proportions and biology, to which they are remarkably constant.

Common Characters of the Group.—Buccal cavity surrounded by lips with short setæ. Within two[1] chitinous teeth. Vulva situated in middle of body.[2]

[1] Some species of Diplogaster, for instance D. fictor Bastian, possess only one buccal tooth.

[2] D. gracilis Bütschli has a "monohysterous" ♀ organ with the vulva a short distance anterior to the anus.

Male with bursa and nine (in one case eleven) pairs of papillæ arranged in manner described above. Spicules slender, with accessory piece.

Synopsis of Group.

(1) Bursa with nine pairs of papillæ: D. longicauda Claus. Bisexual species. Length of ♀ 1000–1300 μ; œsophagus fairly long (one sixth to one seventh of whole length); tail long (one third to one fourth of whole length). Germany.

D. linstowi sp. n. Hermaphrodite species. Length of hermaphrodite 1760 μ; œsophagus short (one ninth of whole length) and tail short (one-seventh). Oviparous at first, but soon became viviparous. Naples.

D. maupasi sp. n. Hermaphrodite species. Length of hermaphrodite 1024–1232 μ; œsophagus (one seventh to one eighth of whole length), tail short (one sixth to one seventh). Oviparous throughout life; 150–300 fertile eggs always laid at early stage of cleavage, and then about as many unfertilised eggs. Norfolk Broads.

(2) Bursa with eleven pairs of papillæ. D. robustus Maupas. Hermaphrodite species. Length of hermaphrodite 2488 μ; œsophagus short (one ninth body length); tail very short (one ninth body length). First oviparous, then viviparous, after laying 150–230 fertile eggs.

In addition to the summary diagnosis above the following characters are distinctive of the two new species.

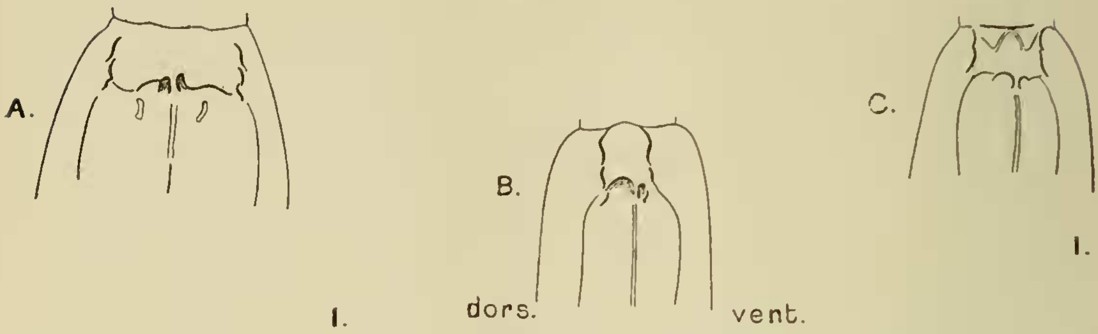
Diplogaster maupasi sp. n. (Text-figs. 1, 4, 5, 6, 8).

Typical measurements of old ⚥:

Total length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.	Length of egg.
1232 μ	608 μ ($\frac{1}{2}$)	152 μ ($\frac{1}{8}$)	176 μ ($\frac{1}{7}$)	5·6 μ

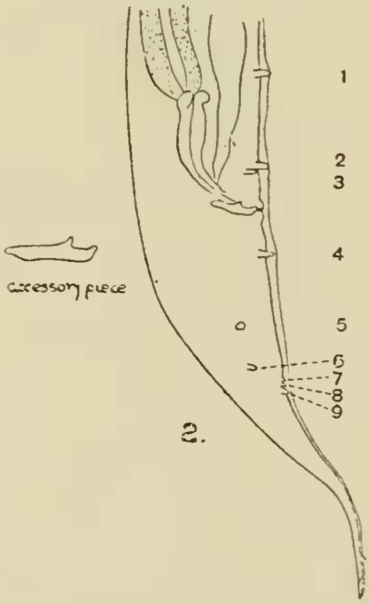
Buccal cavity small, with three indistinct lips, each with a slender seta, often distinguished with difficulty. Hermaphrodite at first lays eggs at long intervals, more frequently later. Males often fairly common. Spicules short, slender, and almost colourless; accessory piece small, in lateral view generally a right-angled triangle, but frequent departures from this type by the rounding of the angles. Number and arrangement of the bursal papillæ strikingly variable.

Text-fig. 1.



It was at first thought that the shape of the buccal cavity was distinctive of species. The accompanying diagram of D. maupasi shows how greatly the state of contraction of the mouth affects the buccal cavity.

Text-fig. 2.



D. linstowi sp.n. (Text-fig. 2).

Typical measurements of old ⚥:

Total length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
1760 μ	840 μ (½)	200 μ ($\frac{1}{9}$)	240 μ ($\frac{1}{7}$)

Buccal cavity large, as broad as deep, with six papillar lips, each with a slight seta not easily seen.

Males with long and slender copulatory spicules and stout accessory piece, elongated and pointed distally (contrast triangular piece of D. maupasi).

Rhabditis Dujardin.

(1) R. gurneyi sp. n. (Text-figs. 9, 10).

Measurements:

	Length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
Old hermaphrodite	1456μ	709μ (½)	243μ ($\frac{1}{6}$)	149μ ($\frac{1}{9}$–$\frac{1}{10}$)

Diagnosis.—Hermaphrodite rather long and slender, tail short. Lips of buccal cavity indistinct, with very minute setæ; buccal cavity narrow and deep. First division of œsophagus thick. Vulva median. Hermaphrodite gland with alternating production of spermatozoa and ova. Spermatozoa of large size. Number of fertile eggs laid up to 800.

Male unknown; probably never produced.

Locality.—In peaty soil, Longmoor Point, Sutton Broad, Norfolk.

(2) R. sechellensis, sp.n. (Text-fig. 3).

Measurements:

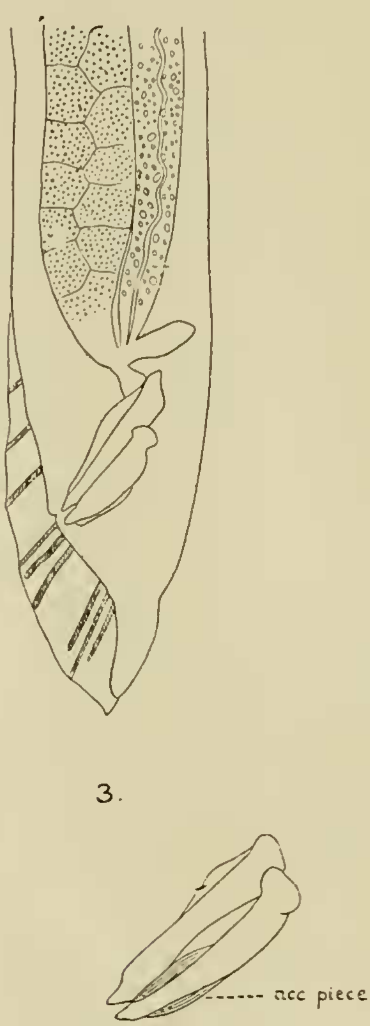
	Length.	Head to vulva.	Head to end of second bulb of œsophagus.	Anus to tail.
Old hermaphrodite	680μ	344μ (½)	128μ ($\frac{1}{5}$)	120μ ($\frac{1}{5}$–$\frac{1}{6}$)

A male measured 496μ in total length.

Diagnosis.—Small Rhabditis of pale, transparent appearance. Lips of buccal cavity indistinct, surmounted by minute setæ, only made out with greatest care. Buccal cavity narrow and deep. Tail of moderate length. In hermaphrodite vulva median. Number of eggs produced small

TEXT-FIG. 3.

A



(150 or less), mother dying before exhaustion of spermatozoa. Males rare, inert. Copulatory spicules short and thick, accessory spicule small and inconspicuous. Bursa supported by nine rays, arranged as in Text-fig. 3.

Locality.—Found in moss from Seychelles; brought back by Professor J. Stanley Gardiner.

Biology in Relation to Methods of Experiment.

To obtain soil-nematodes in large quantities, it is only necessary to place scraps of flesh on samples of rich soil or mould kept moist and warm, and wait till decay has set in. Though the normal nutriment of these animals is presumably associated with the decay of vegetable products rather than decomposing animal matter, the latter prove exceptionally attractive. When once putridity commences, five or six days more suffice for the appearance of very large numbers of rhabdites or diplogasters, generally belonging to one or two species. Before, however, the last remains have vanished, it is probable that other species will have appeared and become dominant, entirely replacing the first kinds, so that an alternation is obtained somewhat similar to the succession of Protozoa in putrefying broth. It seems that the soil contains scattered throughout it numerous encysted larvæ, for, as Maupas has pointed out, when insufficient nutriment is supplied to soil-nematodes, the young larvæ envelop themselves in a thick cuticle, and become rigid and immobile. They are capable, however, of violent contortions, as if for the purpose of freeing themselves from the cyst, and by these movements migrate easily through the soil. The cuticular protection enables them to live uninjured in a dry environment, so that soil, etc., which has been subjected for long periods to fairly high temperatures, will yet yield large numbers of nematodes when treated in the way described above. The power of encystment, and consequently of resisting prolonged desiccation, is confined to the larvæ. Adult worms at once die when a liquid culture in which they are contained is allowed to dry up, and the eggs of these forms are provided only with a thin cuticular envelope, and are incapable of resisting the vicissitudes to which the eggs of parasitic forms like Ascaris are successfully exposed. When, then, animal-matter putrefies on a sample of soil, it is the encysted larvæ which are attracted to its neighbourhood, where they emerge from their cysts and commence to feed and grow rapidly. The rate of increase is very great: a single individual when once it has become mature will in five or six days give rise to one or two hundred, the eldest of which will be beginning to lay eggs. But a short interval then elapses between the migration of encysted larvæ toward the putrid meat and the appearance of the swarms of young worms of the second generation.

It is perfectly easy to keep free-living nematodes in drops of a nutrient fluid, and observe under the microscope every stage of their growth and reproduction. Each of these drop-cultures is contained in a solid watch-glass and secured against evaporation by a vaselined glass cover. Solutions of peptone were adopted as convenient culture media, and used almost exclusively in these experiments. The solutions were first allowed to putrefy till a cloudy growth of bacteria had developed throughout the liquid. So favourable an environment for growth does a peptone solution in this condition constitute, that in four days the eggs laid by a mature hermaphrodite nematode have themselves produced mature individuals. It is only in the presence of great numbers of bacteria, or the substances formed by them, that the nematodes thrive so well. In sterile solutions growth is suspended, and eggs are only laid at long intervals, for apparently nematodes find it difficult or impossible to assimilate peptones in an unaltered condition. It has not been discovered whether digestion takes place by the secretion of juices dissolving the protoplasm of the bacteria, or is merely confined to the absorption of soluble substances present in the culture fluid and prepared by the action of bacteria. If the second alternative be correct, then a parallel is established with the parasitic nematodes which nourish themselves on the dissolved and broken-down food of their host. An easily observable phenomenon of nematodes in culture is the rapid pumping action of the second œsophageal bulb and the rectum, and it may be argued from this that the nutriment obtained from the stream of fluid so constantly passing through the alimentary canal is in the form of easily abstracted soluble substances.

The insignificant development of glandular cells (which are found only in the œsophagus) may be cited against an intra-intestinal digestion of the bacteria, and whatever else its significance may be, the chitinous layer which lines the alimentary canal throughout must prevent an ingestion of bacteria by the endoderm cells themselves in such a way as Colpidium preys upon the bacteria of the soil.

Besides peptone solutions other culture media have been used in the course of experiment. It was found possible to raise two or three successive generations in a saturated solution of gelatin in water, and free-living nematodes matured from the egg in solutions of amides like tyrosin and leucin, but in these cases the growth was so much retarded and the production of fertile eggs so curtailed that only peptone solutions were used for extended experiments.

The temperature at which the cultures were kept varied from about 18° C. in the summer to 12° C. in the winter, though at one period it fell within three or four degrees of zero. The effect of a temperature approaching freezing-point was very marked, and showed itself in the almost entire suspension of growth. Sterility was not induced, but only a very few eggs were laid every day.

Experiments were also made to find the highest temperatures under which life and reproduction could continue. The cultures were placed in a water-bath which could be kept down to 25–30° C. Several individuals of the sixth generation were isolated with the temperature of the bath at 26° C., going up to 28° C. One of these laid forty-three eggs on September 8th. By September 11th these had developed into hermaphrodites of mature size, but although they lived for several days and were apparently in a quite healthy condition, they never produced mature eggs or spermatozoa. The ovary was distinctly seen with small nuclei, but there was no aggregation of yolk. Changes of this kind occurred in the other cultures.

In addition individuals just ready to lay eggs were isolated from the cultures at the temperature of the room and placed in a bath at 26–28° C. Under these conditions the ovary continued to produce large-yolked eggs, and at first these were fertilised and laid, but after they had completed a few divisions they became disorganised. With eggs which later passed from the ovary into the uterus fertilisation did not apparently take effect. No egg-shell was formed, and the uterus became full of an amorphous, yolky mass.

It seems, then, that the limits of reproduction lie in Diplogaster maupasi between 19° C. and 25° C., though life may be continued at slightly higher temperatures. It was found impossible, however, to keep cultures at a constant temperature of over 30° C. The individual worms became rigid and after a short exposure died. It is seen that the free-living nematodes are most sensitive to increased temperature in the egg stage, when they can hardly endure high summer heat. The adult is also likely to succumb at temperatures which must be common in tropical countries at least. The encysted larvæ are probably the most resistant stage, and it must be supposed that these animals depend for their existence in periods of exceptional heat to their capacities for survival in this condition.

The Males of Hermaphrodite Species.

(1) Structure and Organisation.

The male sex in Rhabditis and Diplogaster, as in all nematodes, is sharply discriminated by the relation of the vas deferens to the alimentary canal, and by the well-defined secondary sexual characters, including a membranous bursa for adhesion to the female during copulation, and an arrangement of spicules for insertion into the vulva to facilitate the transference of the spermatozoa.

The males of hermaphrodite species occurring in such small numbers, and apparently taking no part in reproduction, might naturally be expected to show some marked signs of degeneracy in organs other than the reproductive system.

In the Cirripedes we have another clear case of the successful establishment of hermaphroditism in a group in which the sexes were originally separate. Here, too, in hermaphrodite species there is a survival of the male sex, but the individuals which represent it are so degenerate in form and structure as to be described as little more than a bag of spermatozoa, and so reduced in size as to well merit the title of "dwarf males."

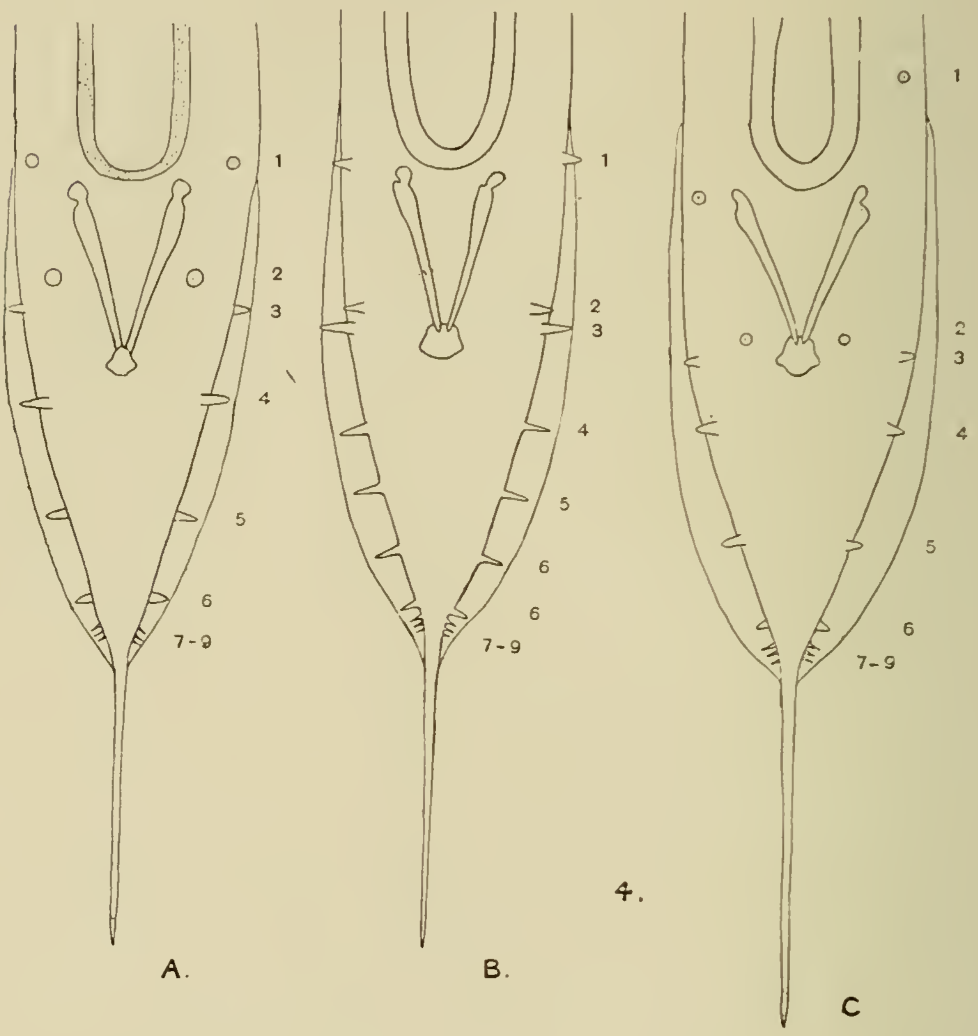
It is, however, a surprising fact that in no particular of structural organisation do the males of hermaphrodite species appear to fall behind those of bisexual nematodes. The conclusions which Maupas reached on this subjects are summed up in the following quotation :

"Ces mâles . . . n'offront rien de particulier et d'anormal. On ne remarque rien dans leur structure et dans leur organisation générale qui puisse les faire considérer comme des animaux mal venus ou mal constitués. Par leur taille, par les proportions de leur corps et par tous les details de leur organisation, ils répondent de tous points au type mâle ordinaire des Rhabditides dioique. Leur testicule luimême est constitué d'une façon absolument normale et, ses produits, les spermatozoïdes, sont palreur forme, leur volume et leur structure absolument identiques a ceux que la glande génitale des femelles produit pendant sa periode d'activité protérandrique."

My own observations show that there is no imperfection of development in the residual males of such species as I was able to study. The spermatozoa were always produced in vast quantities and exactly like those formed by the hermaphrodites. When liberated by pressure from the body of the male, they could be observed to put out amœboid processes like those which Ziegler figures taking up their position in the uterus of Diplogaster longicauda after fertilisation. This observation tends to show that the spermatozoa are physiologically active though the individual which carries them is prevented from playing its part in reproduction, possibly by a defect in nervous organisation.

The experiments of Maupas with Rhabditis elegans showed that on the rare occasions when males do fertilise hermaphrodities, the spermatozoa are perfectly efficacious in the production of embryos. The curious change in the sex-proportions of the offspring of such unions may, however, be

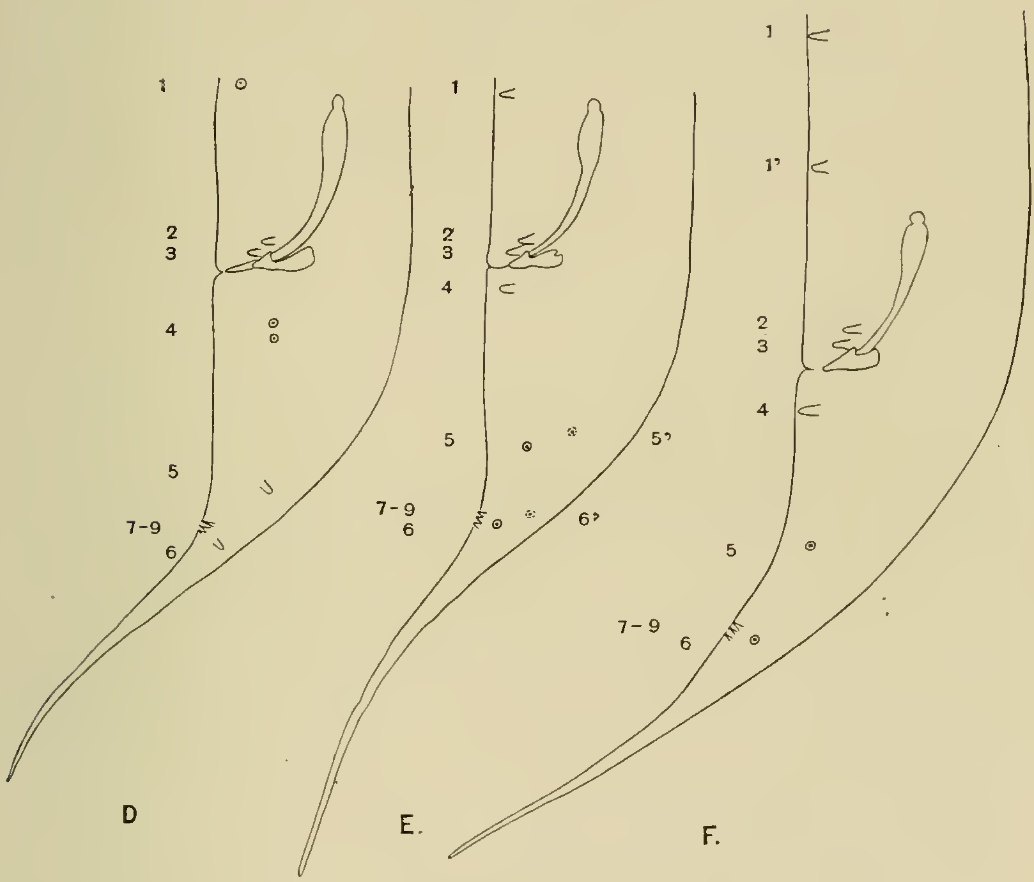
Text-fig. 4.



eventually traced back to some essential difference in the spermatozoa of males and hermaphrodites respectively, which might be revealed by a thorough examination of the spermatogenesis in the two cases.

But though there is no manifest imperfection of organisation in the males of hermaphrodite species, they appear to be sometimes distinguished by extreme variability of the secondary sexual characters. In such specific characters as size and proportions of various parts the males are fairly constant, but the arrangement of the papillæ supporting the copulatory bursa and the shape of the accessory piece of the copulatory spicules show wide differences. When Diplogaster maupasi was first obtained from various

TEXT-FIG. 4.



samples of soil round Sutton Broad, the differences existing between the males found in separate cultures made me conclude that I was dealing with a number of nearly related species. It soon became clear that distinct types of male were not characteristic of each culture, but that even brothers from the same family often exhibited wide differences.

The typical arrangement of the bursal papillæ in Diplogaster maupasi is shown in A, Text-figure 4. Departure from this type was found, however, in almost every other specimen examined. Below are given some of the clearest cases of variation observed in dealing with a comparatively small number (about forty) of males.

(1) There should be normally a pair of papillæ situated exactly opposite the anterior end of the copulatory spicules. One of the most frequent and easily demonstrated variations occurs when one of the pair (or very rarely both) is shifted forward a smaller or greater distance. So marked a case as fig. C was observed two or three times.

(2) A pair of papillæ (4–4′) occurs a short distance posterior to the anus. Only small variations in position were recorded here, but on one occasion a duplication of the papilla of one side was observed (fig. D). (The papilla of the other side was seen on altering the focus, so it was quite evident that the twin papillæ belonged to the same side.)

(3) In the position of papillæ 5 and 6 there is rather frequent variation; they are sometimes nearer together, sometimes further apart. Occasionally it may be seen (when the animal is lying on its back) that the papillæ of the two sides (5, 5′, and 6, 6′) have a tendency to alternate in position (fig. B shows this, but not very well). An example like fig. B was observed once, in which one of the papillæ, either 5 or 6, was duplicated on both sides, and the twin papillæ then shifted apart.

(4) The three small papillæ at the root of the tail (7–9) are rarely replaced by two.

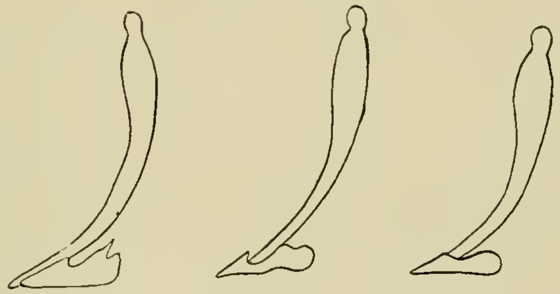
It is only occasionally on examining these animals that a frontal view is obtained, showing the rays of the bursa on both sides. In side views it is often difficult to correctly observe the position of the papillæ. On this account only a few definite cases of variation are referred to above. They were observed in dealing with forty to fifty males.

The accessory piece of the spicular apparatus varied in form in nearly every individual. Three types are figured. The first shows the most typical, in the shape of a right-angled trangle, with an indentation at the anterior angle. In the other two the angles become more and more rounded.

In Rhabditis sechellensis variations in the secondary sexual characters are occasionally found, but are much less numerous than in Diplogaster maupasi. Such variability as was observed was manifested in (1) inequality of the copulatory spicules, and (2) occasional asymmetrical disposition of the rays of the bursa.

The only reference to analogous phenomena which occurs in Maupas' paper is found in his description of Rhabditis guignardi (p. 525). He obtained only two males, but in one of these the copulatory bursa possessed on each side nine

TEXT-FIG. 5.



A. B. C.

supporting rays, in the other only seven. In the latter the remaining rays showed a disposition to fuse with each other, a phenomenon, it may be remarked in passing, which was responsible for the asymmetry of the bursal rays in R. sechellensis. The entire disappearance of two rays is a variation as great as any recorded above for Diplogaster maupasi.

The position and number of bursal papillæ or rays is looked upon as clearly diagnostic of species of Rhabditis or Diplogaster, and as far as I know no striking variation has ever been observed in the bisexual species. The connection of such a variability in the males with their disappearance from the economy of the species is no doubt significant, but it is impossible to offer any explanation of the facts.

(2) Proportions of Males in Hermaphrodite Species.

Another remarkable feature of the males of hermaphrodite species studied by Maupas is their extreme rarity. In only one out of eleven species investigated was he unable to find a male; but in others males were only discovered by organising cultures of very considerable size, containing several thousand mature worms. So while in the majority of species the males were less than 0·1 per cent. of the whole number of adults, the proportion of 4 per cent. to which they rise in Rhabditis marionis affords quite a striking contrast. In Diplogaster maupasi, one of the species obtained from the Norfolk Broads, the ratio of male to female is very much more notable than anything which Maupas records, and does occasionally approach, though remotely, that equality of the sexes which is characteristic of the majority of animal forms. In one large culture the males reached 10 per cent. of the whole (377 ☿, 38 ♂ ♂), and in batches of eggs laid by the same individual up to 30 per cent. (16 eggs, 11 ☿, 5 ♂ ♂; 29 eggs, 23 ☿, 6 ♂ ♂). These instances are, of course, specially favourable, and picked from amongst scores of cultures which did not yield a single male. It is very unlikely that a species will be discovered uniformly consisting of equal numbers of males and hermaphrodites. Southern[1] supposed that in Rhabditis brassicæ he had discovered such a species, but in a culture with which he kindly supplied me I have been only able to find males and females, but no hermaphrodites.

To illustrate the manner of occurrence of the males, I give here an analysis of cultures of Diplogaster maupasi carried on over twenty-five generations, from August, 1909, to January, 1910. The whole series of cultures commenced with a single individual. In every subsequent generation at least one hermaphrodite was isolated just before maturity to carry on the succession. When such an individual had commenced to lay eggs it was removed every day to another

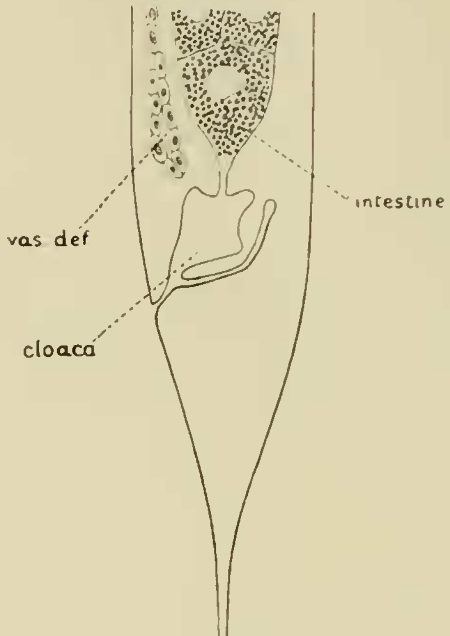
[1] Rowland Southern, "On the Anatomy and Life-History of Rhabditis brassicæ n. sp.," 'Journ. Econ. Biol.,' vol iv, 1909, pp. 90–95.

watch-glass, so that the batch of eggs laid during the preceding twenty-four hours was kept isolated. Each batch was carefully counted to compare with the actual number of individuals attaining adolescence, and in this way records of cultures which gave the actual sex-proportions were distinguished from others in which mortality before maturity obscured the true figures. In any drop culture which contained more than about thirty eggs the crowding which ensued was distinctly unfavourable to the chances of survival.

Precautions were adopted in these experiments to prevent absolutely an association of mature males and hermaphrodites, and so remove any suspicion of cross-fertilisation in the line of descent here followed out. To this end the individual destined to give rise to the next generation was separated before any male had become mature, or else the males themselves were removed from the culture before the last moult, when they were perfectly recognisable as males, but had not yet assumed the spicular apparatus necessary for internal fertilisation.

Both sexes become easily distinguishable a considerable time before maturity by the position of the developing gonad and its duct. In the majority of species of Rhabditis and Diplogaster, the vulva opens at the middle of the body of the female, and the gonad is paired, so that the immature hermaphrodite may be recognised by the symmetrical disposition of the clear ovarian rudiments round the middle point of the body. In the male the rudiment of the testis is situated in the posterior half of the body, so that with a little experience it is easy to distinguish a male, even among a ceaselessly twisting mass of other individuals, by the clear transparent testis running alongside the posterior part of the gut. Sperm-formation begins, it is true, before the last moult. But though the body of the male may contain mature spermatozoa, these can only be conveyed to the hermaphrodite individual by the co-operation of the copulatory spicules and bursa. A young male just before the last moult, at which these latter are developed, is shown in Text-fig. 6. The proximal part of the vas deferens leading into the cloaca does not appear to be yet fully formed. The cloaca is spacious, and is produced on its dorsal surface into a pair of definite pouches in which the chitinous copulatory spicules are formed at the time of the last moult.

TEXT-FIG. 6.



The history of the cultures may be divided into alternating periods, which are distinguished respectively by the frequent occurrence of males and their entire absence. During the first six generations, while these experiments were being prosecuted in Norfolk, the percentage of males was often quite high in batches of twenty or thirty eggs, and the offspring of the majority of individuals contained at least one or two. In addition, the total number of eggs laid by each parent seldom exceeded 130 (150 in one case), and the spermatozoa were not exhausted before death. The seventh and eighth generations were reared away from a laboratory, under conditions which made careful recording difficult. On removing the cultures to Cambridge a new kind of peptone[1] was used for the preparation of a culture-medium, and the behaviour of the nematodes altered considerably with this change. In five generations, from the ninth to the fourteenth, not a single male was produced. The interval elapsing between the arrivals at maturity of successive generations decreased from seven days to four, and the number of fertile eggs laid by each parent rose to between 150 and 300. In every case the life of the individual was prolonged under these more favourable (?) conditions, the period of fertile production being succeeded by another at least as long, during which sterile eggs were laid.

Later, in the fifteenth generation, the peptone used in Norfolk was again tried, and at once males appeared sparingly in the cultures. Later the individuals raised from certain batches of eggs showed a fairly high ratio (e.g. in the nineteenth generation [23] 19 ☿ 4 ♂♂), but in general males were rarer than in the early cultures of August. After another removal at Christmas, 1909, the second period of male production was terminated like the first. It may well be supposed that the alteration of conditions, slight or otherwise, which ensues on changing the place of experiment was directly responsible for the disappearance of the males.

It is not probable, however, that the proportions are controlled by nutrition, for though at first circumstances seemed to indicate that the use for a culture-medium of white peptone acted as a stimulus to male production, from the fifteenth generation onward four series of cultures were maintained, two in white peptone and two in brown (which is the more favourable medium for growth). As mentioned above, males first appeared in the former medium, but in the seventeenth generation they were also observed in brown peptone, and there was no sufficient difference in the figures to suggest which peptone was the better material for the production of males.

[1] In dark brown crystals completely soluble in water.

In the second table a fuller analysis of the experiments lasting over the first six generations is given. An attempt was made to isolate strains, constantly producing high proportions of males, by breeding from a large number of individuals of the same generation. Thus in the third generation a batch of 44 eggs produced 32 ⚥ and 12 ♂♂ (about 28 per cent.) did not, with one exception, maintain those high proportions. One, however, though giving at first hermaphrodites only, laid a batch of 16 eggs of which 11 became ⚥ and 5 ♂♂ (31 per cent.). Nearly all these hermaphrodites were kept for an examination of their progeny, but five individuals, whose records were kept separate, furnished strikingly retrograde results, though males occurred in every case but one. The male ratio was greater in a culture consisting of the offspring of three individuals, reaching 11 per cent. of the whole number. Further selection for the next generation proved equally indecisive.

In the third generation a control series was also established by taking sister individuals from a culture in which only hermaphrodites were represented. The total number of offspring of the five parents selected was 319, of which 302 were ⚥ and 17 ♂♂. This is exactly comparable to the total of 262 ⚥ and 15 ♂♂ produced by the five individuals from a culture with 28 per cent. of males. The individual details are closely similar in the two series.

A brief inspection will serve to show how extraordinarily irregular is the distribution of males in the progeny of any single worm. There is no rule that they should appear at stated intervals or restricting their production to a period or periods of maturity, but on the contrary the appearance of a few males from an early batch of eggs may be followed by a succession of hermaphrodites only and *vice-versâ*; the last eggs may produce males when there have been only hemaphrodites hitherto, or, again, males may occur in several successive batches.

TABLE I.

NOTE. *The figures enclosed in circles represent the number of eggs laid in each batch: those to the right the individuals counted on arrival at maturity or before.*

1st Generation. Offspring of a single isolated hermaphrodite. no males observed.

Generation	Eggs		Hermaphrodites		Males	Eggs		Individuals
2nd ,,	26		⚥		2 ♂♂.			
3rd ,,	(44)	:	32 ⚥		12 ♂♂.			
4th ,,	(25)	:	25 ⚥.					
	(23)	:	9 ⚥.		2 ♂♂.			
	(12)	:	——		.			
	(23)	:	——		.			
	(16)	:	11 ⚥.		5 ♂♂.			
	(9)	:	——		.			
Total	108	:	45 ⚥	:	7 ♂♂.			
5th ,,	(36)	:	32 ⚥	:	4 ♂♂.			
	(33)	:	32 ⚥	:	1 ♂♂.			
	(30)	:	30 ⚥.					
	(34)	:	34 ⚥.					
	(17)	:	16 ⚥	:	1 ♂♂.			
Total	150	:	144 ⚥	:	6 ♂♂.			
6th ,,	(18)	:	12 ⚥	:	1 ♂♂.	(90)	:	55 ⚥ 3 ♂♂.
	(46)	:	——		.	(44)	:	—— .
	(25)	:	——		.	(10)	:	—— .
	(38)	:	——		.			
Total	127							
7th ,,	(18)	:	14 ⚥	:	1 ♂♂	(42)	:	29 ⚥ 1 ♂♂.
	(16)	:	——		.	(27)	:	—— .
8th ,,	(7)	:	7 ⚥.			—	:	51 ⚥ 2 ♂♂.
9th ,,	(40)	:	——.			—	:	26 ⚥.
	(30)	:	21 ⚥.					
	(30)	:	——.					
	(74)	:	26 ⚥.					
10th ,,	(10)	:	10 ⚥.			(35)	:	
	(102)	:	26 ⚥.			(80)	:	31 ⚥.
	(117)	:	73 ⚥.			(48)	:	37 ⚥.
	(14)	:	11 ⚥.			(12)	:	12 ⚥.
Total	243	:	120			175	:	80 ♀

These first six generations were bred in the Sutton Broad Laboratory, Norfolk. For the first generation an infusion of Beef was used. Afterwards two or three varieties of Peptone (Dry, Albumen, Witte's) supplied by Harrington Bros., all of which had substantially the same value as a food stuff.

The 10th–19th generations were bred in the Zoological Laboratory at Cambridge.

TABLE I (CONTD.)

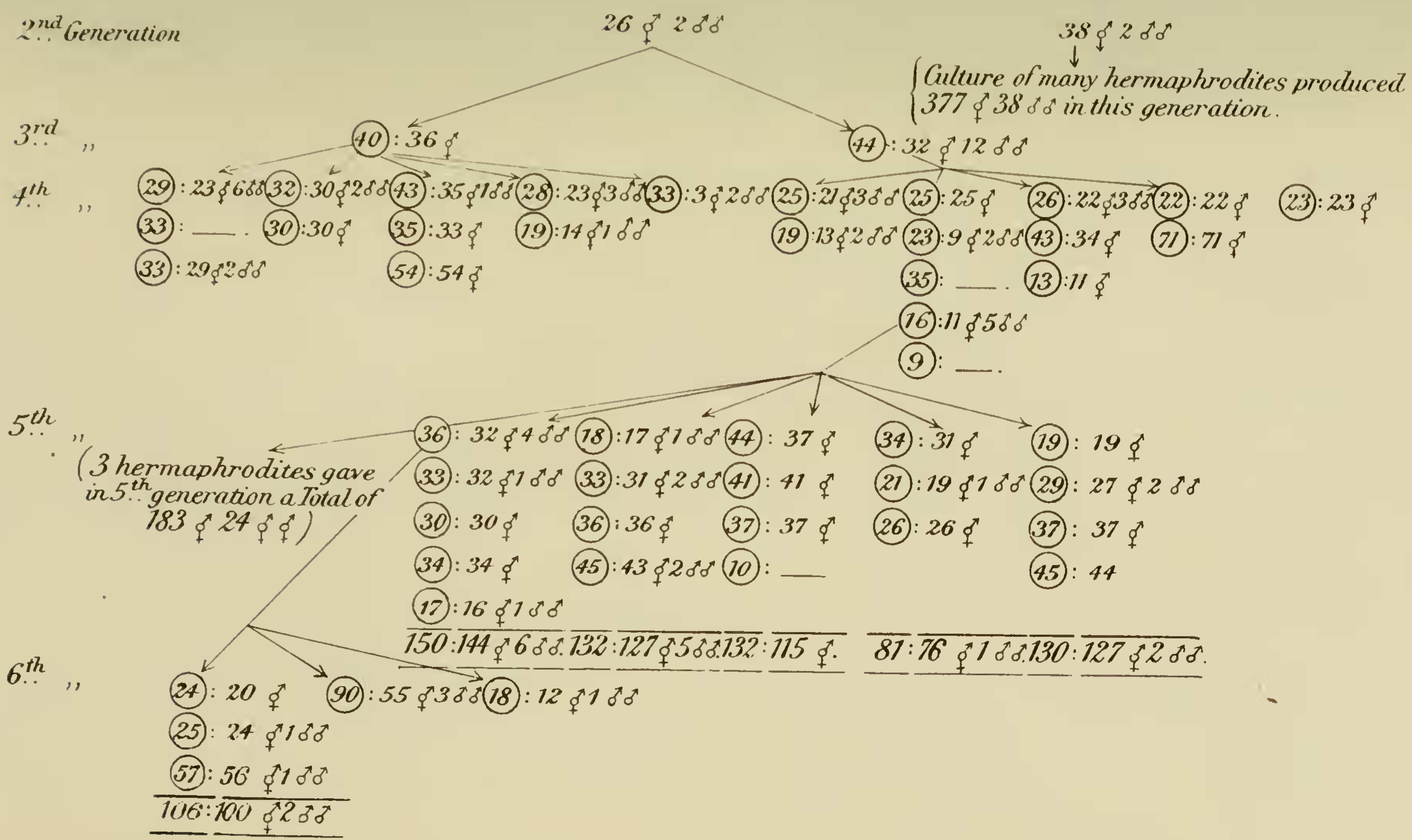
Generation				
11th Generation.	(37) :	35 ⚥	(26) :	19 ⚥
	(60) :	25 ⚥	(39) :	25 ⚥
	(65) :	—	(48) :	48 ⚥
	(20) :	—	(55) :	—
	182 :	60 ⚥	168 :	92 ⚥
12th ,,	(18) :	18 ⚥	(14) :	14 ⚥
	(56) :	46 ⚥	(35) :	10 ⚥
	(38) :	38 ⚥	(29) :	17 ⚥
	(66) :	47 ⚥	(50) :	—
	(68) :	—	(65) :	27 ⚥
	246 :	149 ⚥	193 :	68 ⚥
13th ,,	(40) :	28 ⚥	(18) :	18 ⚥
	(65) :	11 ⚥	(50) :	6 ⚥
	(24) :	8 ⚥	(26) :	16 ⚥
	(56) :	9 ⚥	(49) :	40 ⚥
	(65) :	12 ⚥	(110) :	75 ⚥
	250 :	68 ⚥	(4) :	4 ⚥
			257 :	159 ⚥
14th ,,	(14) :	14 ⚥	(28) :	28 ⚥
	(25) :	6 ⚥	(32) :	12 ⚥
	(32) :	31 ⚥ 1 ♂♂	(35) :	28 ⚥
	(36) :	36 ⚥	(40) :	39 ⚥
	(34) :	—	(41) :	34 ⚥
	(2) :	2 ⚥	(16) :	12 ⚥
	143 :	89 ⚥ 1 ♂♂	192 :	153 ⚥

15th gen.	*Brown Peptone.*		*White Peptone.*		*Brown Peptone.*		*White Peptone.*
			(27) :	20 ⚥			
	(42) :	32 ⚥	(21) :	20 ⚥	(20) :	20 ⚥	*The one selected here was apparently entirely sterile.*
	(26) :	26 ⚥	(45) :	30 ⚥	(18) :	18 ⚥	
	(72) :	40 ⚥	(40) :	31 ⚥	(50) :	50 ⚥	
	(92) :	70 ⚥	(44) :	36 ⚥ 1 ♂♂	(81) :	52 ⚥	
	(31) :	31 ⚥	(41) :	36 ⚥	(30) :	30 ⚥	
	(22) :	22 ⚥	(24) :	24 ⚥	(32) :	30 ⚥	
	285 :	221 ⚥	242 :	197 ⚥ 1 ♂♂	231 :	200 ⚥	

TABLE I (CONTD.)

	Brown Peptone.	White Peptone.	Brown Peptone.	White Peptone.
16th Generation.	(26) : 24 ⚥	(38) : 35 ⚥ 3 ♂♂	(24) : 24 ⚥	(13) : 6 ⚥
	(94) : 22 ⚥	(38) : 8 ⚥	(100) : 42 ⚥	(49) : —.
	(62) : 54 ⚥	(34) : 19 ⚥ 1 ♂♂	(39) : —	(41) : 13 ⚥
	(41) : 36 ⚥	(39) : 39 ⚥	(61) : 35 ⚥	(56) : 56 ⚥
	(26) : 26 ⚥	(29) : 29 ⚥	(27) : 19 ⚥	(38) : 38 ⚥
	249 : 162 ⚥	(56) :	(41) : 2 ⚥	(20) : 20 ⚥
		234 : 130 ⚥ 4 ♂♂	292 : 122 ⚥	217 : 133 ⚥
17th ,,	(24) : 24 ⚥	(38) : —.	— : 33 ⚥	(52) : —.
	(55) : 34 ⚥	(64) : 51 ⚥	— : 20 ⚥	(54) : —.
	(60) : 35 ⚥	(50) : 50 ⚥	— : 31 ⚥	(39) : 39 ⚥
	(48) : —.	(46) : —.	— : 26 ⚥	(15) : —.
	187 : 93 ⚥	(29) : —.	— : 15 ⚥ 3 ♂♂	(2) : 1 ⚥
		(27) : 23 ⚥	: 125 ⚥ 3 ♂♂	162 : 40 ⚥
		254 : 124 ⚥		
18th ,,	(39) : 36 ⚥	(33) : 33 ⚥	(30) : 24 ⚥	(25) : 23 ⚥ 2 ♂♂
	(20) : 20 ⚥	(16) : 12 ⚥	(19) : 17 ⚥	(15) : —.
	(26) : 17 ⚥	(11) : 11 ⚥	(96) : 40 ⚥	(2) : —.
	(52) : 28 ⚥ 1 ♂♂	(25) : —	(30) : 14 1 ♂♂	42 : 23 ⚥ 2 ♂♂
	(13) : 11 ⚥	(29) : 28 ⚥ 1 ♂♂	(2) : 2 ⚥	
	150 : 112 ⚥ 1 ♂♂	(12) : 12 ⚥	177 : 97 ⚥ 1 ♂♂	
		126 : 96 ⚥ 1 ♂♂		
19th ,,	(24) : 19 ⚥	(26) : 26 ⚥	(17) : 13 ⚥	(23) : 19 ⚥ 4 ♂♂
	(22) : 10 ⚥	(15) : 15 ⚥		(8) : 8 ⚥
	(16) : 16 ⚥	(11) : —.		(24) : 23 ⚥ 1 ♂♂
	(16) : 8 ⚥	(38) : 19 ⚥		(76) : 74 ⚥
	(58) : 51 ⚥	(88) : 88 ⚥		(14) : 13 ⚥ 1 ♂♂
	136 : 104 ⚥	(21) : 21 ⚥		145 : 137 ⚥ 6 ♂♂
		199 : 169 ⚥		

TABLE 2.


2nd Generation
26 ⚥ 2 ♂♂
38 ⚥ 2 ♂♂
Culture of many hermaphrodites produced 377 ⚥ 38 ♂♂ in this generation.
3rd "
40 : 36 ⚥
44 : 32 ⚥ 12 ♂♂
4th "
29 : 23 ⚥ 6 ♂♂
32 : 30 ⚥ 2 ♂♂
43 : 35 ⚥ 1 ♂♂
28 : 23 ⚥ 3 ♂♂
33 : 3 ⚥ 2 ♂♂
25 : 21 ⚥ 3 ♂♂
25 : 25 ⚥
26 : 22 ⚥ 3 ♂♂
22 : 22 ⚥
23 : 23 ⚥
33 : ——
30 : 30 ⚥
35 : 33 ⚥
19 : 14 ⚥ 1 ♂♂
19 : 13 ⚥ 2 ♂♂
23 : 9 ⚥ 2 ♂♂
43 : 34 ⚥
71 : 71 ⚥
33 : 29 ⚥ 2 ♂♂
54 : 54 ⚥
35 : ——
13 : 11 ⚥
16 : 11 ⚥ 5 ♂♂
9 : ——
5th "
(3 hermaphrodites gave in 5th generation a Total of 183 ⚥ 24 ⚥ ⚥)
36 : 32 ⚥ 4 ♂♂
18 : 17 ⚥ 1 ♂♂
44 : 37 ⚥
34 : 31 ⚥
19 : 19 ⚥
33 : 32 ⚥ 1 ♂♂
33 : 31 ⚥ 2 ♂♂
41 : 41 ⚥
21 : 19 ⚥ 1 ♂♂
29 : 27 ⚥ 2 ♂♂
30 : 30 ⚥
36 : 36 ⚥
37 : 37 ⚥
26 : 26 ⚥
37 : 37 ⚥
34 : 34 ⚥
45 : 43 ⚥ 2 ♂♂
10 : ——
45 : 44
17 : 16 ⚥ 1 ♂♂
150 : 144 ⚥ 6 ♂♂. 132 : 127 ⚥ 5 ♂♂. 132 : 115 ⚥.
81 : 76 ⚥ 1 ♂♂. 130 : 127 ⚥ 2 ♂♂.
6th "
24 : 20 ⚥
90 : 55 ⚥ 3 ♂♂
18 : 12 ⚥ 1 ♂♂
25 : 24 ⚥ 1 ♂♂
57 : 56 ⚥ 1 ♂♂
106 : 100 ⚥ 2 ♂♂

Sexual Instincts of the Males.

Maupas' conclusion that the residual males could not take any part in the production of offspring is expressed in the following words: "Mais si ces animaux examinés dans leur structure et leur morphologie, représentent des mâles vrais et complets, il n'en plus de même lorsqu'on les étudie au point de vue de leurs facultés et de leur activité sexuelles . . . ces mâles ont a peu près totalement perdu tout instinct et tout appétit sexuels. . . . Nous trouvons en présence d'une decadence psychique non concomitante avec une regression morphologique."

This conclusion is supported by the inert behaviour of the males, the fact that they are never seen in copulation with hermaphrodites, but principally by the results of a fairly full series of experiments which Maupas made with males and hermaphrodites which had exhausted their own stock of spermatozoa. These conclusively showed that the males have almost, but not quite, lost their sexual instinct. One species alone stands as an exception. In Rhabditis marionis at various times cultures containing in the aggregate 28 hermaphrodites and 42 males were kept under observation. Since all the spermatozoa of the hermaphrodites were exhausted, any production of developing eggs was plainly due to the intervention of the male, and thus a measure of the activity of this sex was obtained. Fertile eggs were laid by 13 individuals to the total number of 150–200, and all these produced hermaphrodites. This species is one of those for which Maupas described a partially developed hermaphroditism, and the author himself regarded it as specially significant that in such a form the male should be less degenerate.

The most complete series of experiments was made with Rhabditis elegans. Here, in twelve cultures, a total of 139 hermaphrodites with their own sperm exhausted and males were associated. Only six of the hermaphrodites were actually fertilised, a proportion which illustrates exceedingly well the sexual inactivity of the males. The chief point of interest lies in the constitution of the offspring of these six individuals. The young produced numbered 274, and of these 147 were hermaphrodites and 127 males. So numerical equality of the sexes is secured in this species by cross-fertilisation, a result in striking contrast to that obtained when R. marionis was the subject of investigation. No permanent effect was produced on the heredity of sex, for when 38 of the hermaphrodites obtained by fertilisation by males were employed as parents for the next generation, 2964 individuals were produced, of which only 7 were males, but the rest hermaphrodites.

Further evidence of the psychical decadence of the males was secured in other species. Though nearly 100 males were employed belonging to five species only a single successful case of re-fecundation was observed, and in this (Rhabditis duthiersi) the fertilised eggs gave 70 hermaphrodites and 1 male.

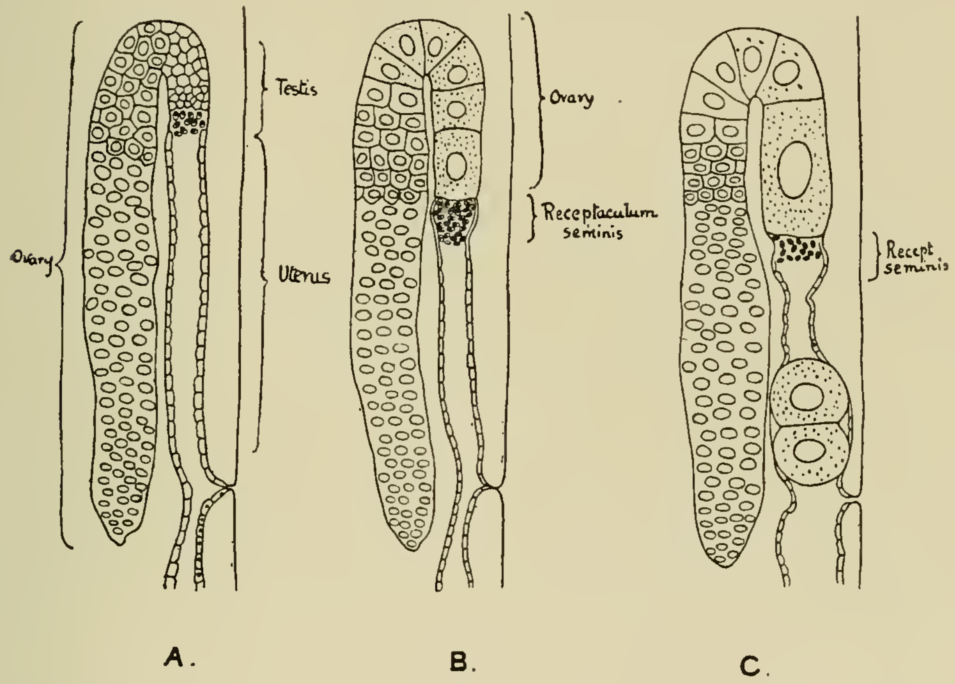
The Hermaphrodites in Hermaphrodite Species.

(1) The Hermaphrodite Glands in Rhabditis and Diplogaster.

In Rhabditis sechellensis the structure and development of the reproductive glands exactly correspond to the description which Maupas gives of R. elegans and R. dolichura. Though no new details can be given, it will be convenient to summarise the changes which the hermaphrodite gland goes through before oviposition commences in any of the above three species. The three diagrams which illustrate the description are partly after my own drawings for R. sechellensis, but closely follow Maupas' sketches of R. dolichura in Plate XXI, figs. 7A, 7B, and C.

The hermaphrodite organ is double, its two divisions being of equal development, and joining at the short and indefinite common vagina. Each division is U-shaped, and consists of a uterus, which extends from the vagina to within a short distance of the bend of the tube, and an ovo-testis, occupying the proximal part of the ventral limb and the whole of the dorsal limb. In individuals examined some hours before the first egg is laid the whole of the ovo-testis appears to consist of cellular elements of nearly equal size, which possess definite boundaries near the bend, but merge into a syncytium distally. The anterior testicular region is indicated by the more regular polygonal form of a comparatively narrow belt of spermatocytes which succeed the uterus. The young egg-

Text-fig. 7.

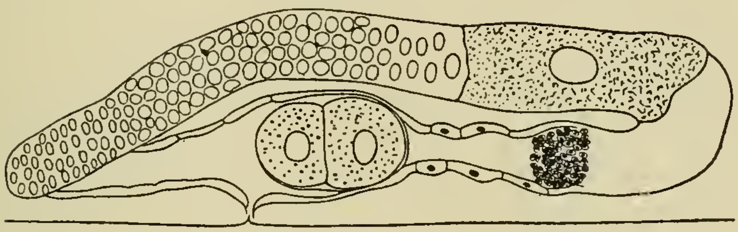


cells which come next are all of small size, and can hardly be distinguished from the male cells. Text-fig. 7, A represents a stage where the testis has begun to function, and several spermatozoa have been formed in the anterior part of the testis.

In the second stage (B) sperm formation is in full activity, or may even be completed by the conversion of all the spermatocytes into spermatozoa. The female part of the gland now begins to show functional activity by the growth of the oöcytes most anteriorly situated. The width of the gonadial tube is so small in comparison with the size of the egg that the growing oöcytes are arranged in a linear series. The oöcyte nearest maturity is just posterior to the sperm-forming region, and behind it is a line of developing egg-cells showing the stages of growth from the scarcely differentiated oögonia. The spermatozoa as fully formed are small circular discs, capable of amœboid movements when effecting fertilisation. They remain in the region of the gland where they were formed, so that what was testis in the first stage becomes receptaculum seminis in the second. In its formation, since the spermatozoa occupy a much smaller bulk than the spermatocytes, the receptaculum seminis shortens considerably; its epithelium is of course the investing layer of the testis. The spermatozoa are now so disposed that the ripe ovum can pass out of the ovary and through the receptaculum seminis without its motion being impeded. During its passage a single spermatozoan fuses with the egg-cell and brings about fertilisation. The fertilised egg immediately becomes enveloped by a cuticular shell, and lies for some time in the uterus undergoing segmentation before it is finally ejected to the exterior by the pressure of eggs from behind (Text-fig. 7, c.). The formation of ripe eggs after the first is perfectly regular, and fertilisation occurs in every case. Since, then, the whole quantity of spermatozoa is formed before the first egg is ready for fertilisation, it follows that a limit is set to the number of fertile eggs it is possible to produce, and as a matter of fact this limit is reached at a comparatively early point in maturity. When the receptaculum seminis is completely emptied of its spermatozoa eggs still continue to be laid at a uniform rate, though they never develop to larvæ.

In Diplogaster maupasi (Text-fig. 8) events follow a very similar course. There is, indeed, one difference in detail during the early periods of egg-laying which may be briefly mentioned. The proximal limb of the gonad is shorter, the distal longer than usual. The former is entirely occupied by the uterus and testicular region, and the ovary is confined to the distal limb. Possibly in accordance with this shortening there is no linear succession of eggs increasing regularly in size in the anterior part of the gland, but each egg grows and reaches its full size before the one next in order begins to differentiate itself in size from the other oögonia. After an egg has passed out of the ovary and been fertilised, a period of some length elapses before the next finishes its growth in the ovary and travels through the receptaculum in its turn. It is only in the early stages, however, that oviposition is a slow process, for as the period of maturity advances, the zone of egg-maturation increases in length, and oögonia are able to start their growth long before the ovum in front is

Text-fig. 8.



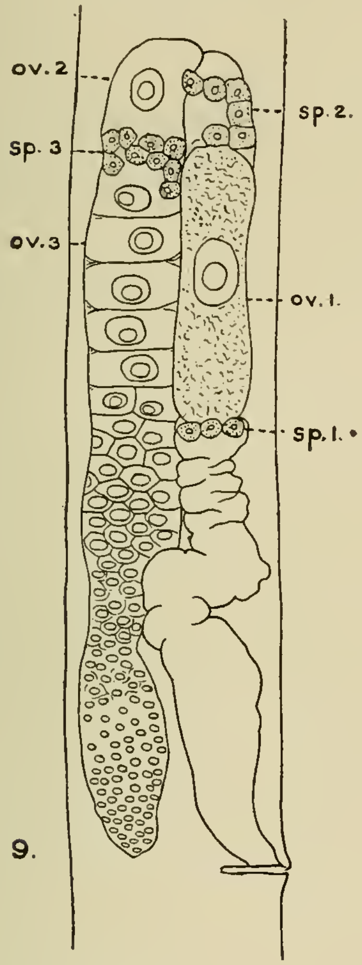
ready to be fertilised. The deliberate character of egg-production in D. maupasi is responsible for the fact that few individuals are seen with more than a single pair of eggs contained in their uteri.

Rhabditis gurneyi.—When this species was first examined large numbers of adult individuals were obtained from cultures of decaying flesh. Amongst these a few were seen which, judging by their size, had only just attained maturity, but whose uteri and vaginæ were occupied by disorganised eggs, as in hermaphrodites, which have exhausted their stock of spermatozoa. It was at first supposed that this was such a species as Rhabditis marionis (cf. Maupas, p. 512), in which a small number of females producing eggs only occur together with the hermaphrodites. When, however, young immature worms were isolated, they were often seen to a stage, sometimes extending over several days, during which eggs passed into the uterus and degenerated. Later, however, the amorphous egg material was expelled and its place taken by fertile eggs which continued to be produced in large numbers. In this species, one could easily see, the hermaphroditism was not protandrous, but the formation of spermatozoa was sometimes delayed till a number of eggs had ripened. In some cases, it is true, fertile eggs are produced from the first onset of maturity, and at first sight there is nothing to distinguish such forms from the typical protandrous hermaphrodite found in other species. But beside such an introductory period of infertility, there may be later interruptions of egg-production, which indicate a failure of the stock of spermatozoa. Frequently this is but temporary, and the worm begins again to lay fertile eggs. So short sometimes is the duration of sterility that it is indicated only by the ejection of one or two disorganised eggs, and very often only one gonad contains a supply of spermatozoa while they are lacking in the other.

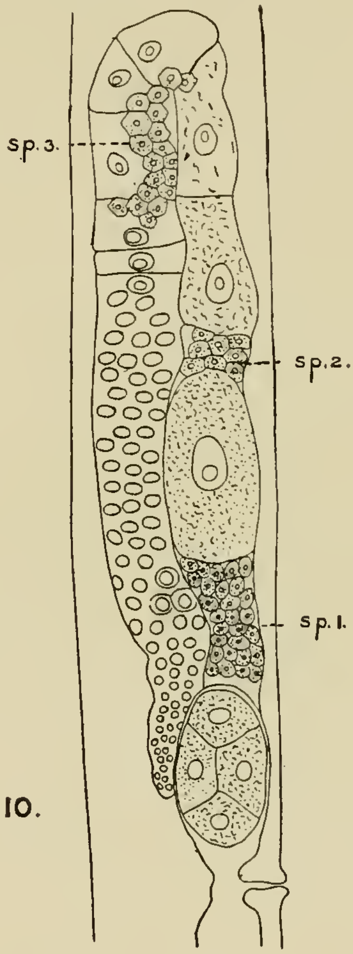
It is, then, suggested by the culture observations, and fully borne out by examination of the glands under high powers of the microscope, that eggs and spermatozoa come to maturity more or less alternately throughout the period of reproductive activity.

Structure of the Gland.—In the general form of the reproductive glands of R. gurneyi there is no departure from that described above for other species of the genus. At various periods of development the arrangement of the histological elements differs rather widely from the typical protandrous gland. Text-fig. 9 shows part of the reproductive organ of a hermaphrodite which has just attained maturity. It will be seen that reproductive activity commenced with the formation of a very small number of spermatozoa (*sp.*[1]). And after the maturation of a single egg (*ov.*[1]) a more numerous succession of spermatozoa (*sp.*[2] and *sp.*[3]) was produced, only briefly interrupted by the appearance of another single egg (*ov.*[2]) which has not yet reached the limit of its growth. After this, a prolonged period of egg-formation appears likely, for posterior to the spermatozoa there is a single row of developing egg-cells (*ov.*[3]) gradually diminishing in size and quantity of yolk, till in the middle of the limb the ovary becomes an undifferentiated syncytium. In this gonad

TEXT-FIG. 9.



TEXT-FIG. 10.



we have at one time the evidences of three alternations of male and female activity within a very limited period.

In the second individual figured (Text-fig. 10) maturity is rather further advanced. The results of the early activity of the gonad are large numbers of spermatozoa and a few eggs. A series of developing ova now promise a long period of female productivity. There is an interesting departure from the appearance of developing sperm-cells and egg-cells in successive belts, for here cells lying side by side may give rise respectively to spermatozoa and eggs. In one case the sperm-cells seem to have been actually formed at the expense of the ovum. The early maturation of the spermatozoa will be noticed here, which terminates while young egg-cells forming from a mother-cell of the same age have only completed the first stages of their growth.

(2) The Fertility of the Soil-nematodes.

The hermaphrodite species of Rhabditis and Diplogaster are distinguished from the bi-sexual, as Maupas points out, by their lesser fertility, a character which indicates the incompleteness of the hermaphroditism. In eleven of the twelve species investigated by Maupas the number of fertile eggs laid by a single hermaphrodite individual varied between 200 and 250, while in the twelfth (Rhabditis guignardi) the limit of production rose to 500 or 520. Maupas states that the female of a bi-sexual species is, on the other hand, capable of laying 700 to 800 fertile eggs. The low fertility of the hermaphrodites is due to the insufficiency of the supply of spermatozoa, for if to the number of fertilised eggs be added that of the unfertilised eggs laid when the male gametes are exhausted, it may be seen that a hermaphrodite produces as many eggs as the female in a bi-sexual species. Individuals producing 200–250 fertilised eggs will afterwards lay two or three times as many unfertilised,[1] so that the total equals the figure given for the bi-sexual species.

Fertility, then, in these hermaphrodites is entirely controlled by sperm-production, and probably the actual number of spermatozoa formed in an individual is given or very closely indicated by counting the eggs laid which develop into larvæ. In these experiments the eggs laid by each parent were counted every twenty-four hours from the beginning of maturity onwards, and the mother then removed to a fresh drop of peptone. Usually after about six days of active ovi-

[1] Maupas. loc. cit., p. 587.

position the spermatozoa become exhausted, but it is difficult to observe exactly when the limit has been reached, because the first laid unfertilised eggs undergo a kind of incipient parthogenetic development. Such eggs possess a shell like fertilised eggs and they complete a few divisions, but the blastomeres are more regular and equal than in normal segmentation; the egg-substance appears greatly shrunk, so that a wide space occurs between it and the egg-shell.

An examination of the table of descent of Diplogaster maupasi will show how widely the fertility varies in a single species even under apparently uniform conditions. A few entries may be specially quoted here for comparison, each pair of individuals being taken from the same generation of nearly related strains and supplied with the same nourishment:

(1) 12th generation October 20th–25th, 257 eggs.
12th generation October 18th–22nd, 153 eggs.

(2) 14th generation October 25th–31st, 143 eggs.
15th generation November 1st–5th, 285 eggs.
In this case a parent with low fertility gave in the next generation exceptionally prolific offspring.

(3) 14th generation October 25th–31st, 192 eggs.
15th generation November 1st–6th, 229 eggs.

Other cases fall within the wide limits indicated above, so that it may be concluded that under favourable conditions a hermaphrodite individual of D. maupasi will lay 140–290 eggs. It is not pretended that such figures as these prove that it is impossible to select strains characterised by high and low fertility respectively, but as far as my observations go, there is a fluctuating variability, not governed by the laws of descent nor always directly traceable to minor changes in the environment.

The influence of external conditions is, however, very great, and especially is this the case with nutrition. In peptone solutions of every kind, the number of eggs laid depends upon the development of bacteria in the culture-medium. When the peptone is fairly sterile the nematode only lays eggs at long intervals, and eventually dies when only a score or so of eggs have been expelled from the uterus. In such a case of course the diminution in fertility is due to the small amount of nourishment supplied to the ovary, which is only enabled to produce a limited number of eggs. When a cloudy film of bacteria is seen at the bottom of the culture-drop the conditions are exceptionally favourable for the growth of the nematodes, and fertile eggs are laid rapidly till the spermatozoa are exhausted. If, instead of peptone, a saturated solution of gelatin be used as a culture-medium, a very different effect is produced. For the first day or so after a worm is moved from a peptone solution into gelatin the rate of egg-production is fairly maintained, but afterwards it sinks very low indeed, though the life of the parent and the period of fertility is much longer than that of individuals in peptone. Thus, for instance, for two hermaphrodites of the same generation bred in peptone but kept during maturity in peptone and gelatin respectively, the following figures were obtained :

(1) Peptone.	(2) Gelatin.
Sept. 2nd–4th, 28 eggs.	Sept. 2nd–4th, 19 eggs.
,, 4th–5th, 32 ,,	,, 4th–15th, 17 ,,
,, 5th–6th, 21 ,,	
,, 6th–7th, 20 ,,	
Total for 5 days 101 eggs	Total for 13 days 36 eggs

When a second generation of Diplogaster maupasi is raised in gelatin, when about twenty fertile eggs have been produced the uterus contains sterile disorganised ova. It appears from this that the effect of the substitution of gelatin as a foodstuff is not merely to curtail the formation of eggs in the ovary, but also to very considerably limit the number of spermatozoa produced.

Though under favourable conditions the average fertility varies between two and three hundred in the majority of species now known, there are undoubtedly some which normally produce a very much smaller number of offspring. In the summer of 1907 I had under observation a species of

Rhabditis from the neighbourhood of Cambridge which I cannot adequately describe from the notes taken at the time It was remarkable for the very small proportion of fertilised eggs laid by each individual. In one family six hermaphrodites were selected before maturity, and their fertility compared. In each case the separate numbers represent the eggs laid in a day, and those in brackets the total of fertile eggs:

A 7, 8, 1, 1, 7=[24] B 9, 10, 4, 4=[27] C 9, 9, 3, 2=[22]
D 7, 4, 12, 8, 2=[33] E 9, 7, 7, 5, 3=[31] F 7, 6, 1, 1=[15]

These cultures were carried on in July. Others, began later in August, gave rather higher numbers, e.g.:

A 1, 7, 13 (and 2 unfertilised eggs), 12, 1=[34]
B 14, 3, 8, 6, 5=[36]
C 15, 17, 11=[43]
D 8, 10, 8, 5, 2=[33]

In A of this second series it will be noticed that the succession of fertilised eggs was interrupted temporarily, but whether this was due to a retarded production of spermatozoa, as in Rhabditis gurneyi, or to some other cause, was not discovered. It is much to be regretted that no trustworthy observations on the occurrence of males were made, for a species like this in which the hermaphroditism is of such an apparently recent and inefficient type, should, according to Maupas' conclusions, possess a very large proportion of males, which was not, however, observed. It is hoped that the species may be rediscovered and this point investigated again.

Rhabditis coronata Cobb, which was investigated by Maupas (pp. 537–541) and shown to be a protandrous hermaphrodite, is probably a similar form with very low fertility. No figures are given of the total of eggs laid, but it is mentioned that an isolated hermaphrodite only laid six eggs in twenty-four hours, and that in general eggs were laid very slowly. An interesting feature shown in Maupas' drawing of the species (Pl. XXI, fig. 8) is the small size of the ovarian part of the gland, which might well account for a restricted egg-production. In the Cambridge species of Rhabditis, on the other hand, the early sterility was certainly due to the extremely small number of spermatozoa. The length of the ovary was proportionately as great as in other species of the genus.

Rhabditis gurneyi, in contrast to the two species last discussed, is a free-living hermaphrodite nematode which has departed from the protandrous hermaphroditism, which we regard as the earliest development from the bisexual state. In consequence it far surpasses others of its kind in fertility. The spermatozoa are of unusual size, and possibly because of the difficulty of providing sufficient space to store a sufficient number at once, they are produced alternately with eggs throughout a great part of the period of reproductive activity. As a result of this adaptation each individual is capable of laying as many eggs as a bisexual female, which frequently has its supply of spermatozoa replenished by copulation.

It must be remembered that in many cases the hermaphrodites of this species only produce unfertilised eggs in the initial period of oviposition which represent a total loss to the organism. When once this critical period has been passed, and a sufficient supply of spermatozoa established, fertile eggs are produced at the rate of 60–80 each day, or distinctly faster than in the case of Diplogaster maupasi and others.

For figures to illustrate the fertility of Rhabditis gurneyi the following case is given. From the offspring of a single individual six immature hermaphrodites were selected. When maturity was reached the eggs laid every twenty-four hours were counted, and the parent removed to a fresh culture drop in the manner described above for Diplogaster maupasi. The dates in each case mark the period over which oviposition continued.

(A) September 6th–17th, 525 fertile eggs.

(B) „ 7th–17th, 686 „

The figures here are not complete, for the culture dried up while the parent was still laying fertile eggs. When 343 had been produced a prolonged failure of spermatozoa, lasting twenty hours, occurred in one of the glands, so that 16 unfertilised eggs were laid with egg-shell, and the uterus beside blocked by disorganised egg material, while the other produced 40 fertilised eggs. After this interval developing eggs were counted to the number of 300.

(C) September 7th–12th, 168 fertile eggs.
(D) „ 9th–20th, 730 „
(E) „ 7th–17th, 362 „
(F) „ 7th–10th, 81 „

Out of the six individuals two laid about 700 eggs each, and though the figures obtained from the others show a high variability, this is partly to be explained by the very marked influence which even a slightly unfavourable change in the conditions can exert on sperm production. In cultures where several individuals are crowded together, it is noticeable that very few eggs are laid, and that the uterus of the worms speedily becomes crammed with disorganised eggs, showing that the sterility is caused by the failure of the male, not the female gametes.

In conclusion, it must be stated that the hermaphrodite species are apparently as successful as the bisexual species in the struggle for existence, for they are found in equal, or sometimes in greater abundance in nature. Evidently, though the means of dispersal of the species is limited by their generally low fertility, an advantage which more than counterbalances is secured by the self-fertilising capabilities of each individual.

(3) Partial Hermaphroditism.

It is here proposed to examine the description of certain species which are said to form a genuine link between the bisexual and hermaphrodite species. The species which Maupas deals with are Rhabditis marionis, R. duthiersi, and R. viguieri.

(1) R. marionis.—A single hermaphrodite kept under observation was found to lay only 129 fertile eggs, while

other individuals of the same species produced about 250 before their spermatozoa became exhausted. A closer examination of a similar hermaphrodite led to the discovery that spermatozoa were only produced in one genital gland; from the other only unfertilised eggs were traced. In half its reproductive system the animal was hermaphrodite, in the other female. A few individuals were also noticed in which both genital glands apparently gave rise to eggs alone and never sperm. The species is thus constituted of—(1) pure females (occurring very rarely); (2) individuals with one ovary and one ovo-testis; and (3) full hermaphrodites forming the majority of the society. No mention is made of any variation in fertility among this latter class, but we are led to believe that all individuals fall into one or other of three sharply marked categories, according to the condition of their gonads. In the light of the results recorded above for other species this seems so remarkable that I think this case should if possible be re-examined.

(2) Rhabditis duthiersi.—Three hermaphrodites were observed, each producing fertilised and sterile eggs simultaneously, and it is suggested that these were possibly semi-hermaphrodites of the type described as occurring in R. marionis. It may, however, be pointed out that in R. gurneyi individuals are found with a similar appearance when the formation of spermatozoa is retarded and does not commence simultaneously on the two sides.

(3) Rhabditis viguieri.—In this species the proportion of males was the largest met with by Maupas (though falling far short of some of the records for Diplogaster maupasi). Males formed 4 per cent. to 5 per cent. of the total in large cultures, and it is almost certain that the proportion would have been larger if single individuals had been selected for cultures.

Of the other individuals some were females, which, when isolated, never produced offspring, but when united with males laid fertile eggs. The larvæ from such unions, it is to be regretted, were not kept. Hermaphrodite forms were in a substantial majority, and it may be useful to quote Maupas' words as to the relative frequency of the three kinds of forms : "Les females non-hermaphrodites mais simplement unisexuées sont également très fréquentes. Il ne suffisait, en effet, de placer sous le microscope une dizaine de femelles prises au hasard pour en recontrer une ou deux unisexuées. Les femelles simplement unisexuées y sont même plus nombreuses que les mâles qui les fécondent sans difficulté. En résumé, chez cette espèce les mâles encore relativement nombreux paraissent avoir conservé leur instinct sexual intact."

It is evident that this species, could it be re-discovered, would form a most interesting subject of study. A precise investigation of the comparative frequency of females and hermaphrodites, and in particular of the relative effects of self- and cross-fertilisation on the sexual constitution of the offspring, would prove of the utmost value.[1]

(4) The Nature of Hermaphroditism in the Nematoda.

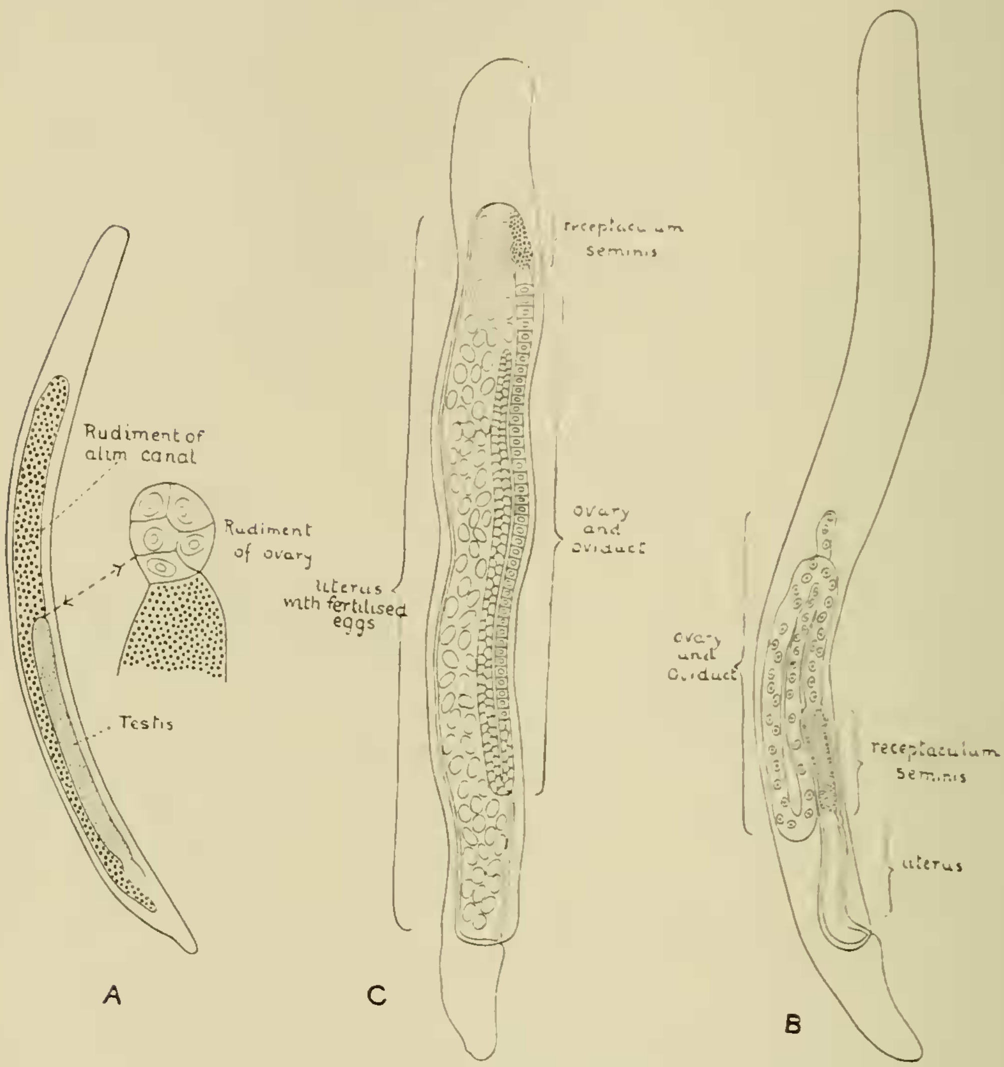
The evidence that the hermaphrodites described by Maupas and myself represent the females of bisexual species, in which a part of the gonad has been given over to the formation of spermatozoa, is, indeed, overwhelmingly strong. Hermaphrodites and females are identical in general anatomy, and the arrangement and form of their gonads are strikingly similar. Then, too, there exist a series of species showing the development of hermaphroditism from small beginnings in species where the ratio of fertilised eggs to unfertilised is very small, until in Rhabditis gurneyi the number of spermatozoa is almost equal to that of the eggs they are required to fertilise. Lastly, there are, apparently, species like Rhabditis viguieri which have not yet decided between bisexuality and hermaphroditism, and present an assemblage of pure

[1] In Diplogaster maupasi, though careful watch was kept only one hermaphrodite was found which failed to develop spermatozoa (see Table I, fifteenth generation).

females, males and hermaphrodites, in cultures probably derived from nearly related individuals.

Similarly, the various species may be arranged in gradation, to show the suppression of the male sex. In Diplogaster maupasi the males occur occasionally in such proportions as

TEXT-FIG. 11.



to recall their original numerical equality with the female sex. But this species, in the majority of cultures and most others, at all times produces males in exceedingly small numbers. Finally, in Rhabditis gurneyi the male has possibly entirely disappeared, though of this it is difficult to adduce positive proof.

There are, however, some indications that it is not the female alone which is capable of developing a hermaphrodite gonad. In Rhabditis elegans Maupas records (pp. 491–492, Pl. XVII, fig. 2) the occurrence of large egg-like cells in the testis. A similar phenomenon has frequently been recorded as characteristic of the normal male gonad in Crustacea (Orchestia), and in other Crustacea the appearance of eggs in the testis, without doubt to be attributed to the indirect action of parasites, is so definitely associated with the development of female secondary sexual characters as to indicate a change to hermaphroditism. In Rhabditis elegans the phenomenon is very slightly manifested, but there are indications that a very much more complete change is imposed on the male of Bradynema rigidum, a nematode parasitic in the body-cavity of the beetle Aphodius fimetarius.[1] This animal is so adapted for its parasitic life that mouth and anus have disappeared, and the alimentary canal, in the larva represented only by a single column of cells, has left not the slightest trace in the adult. In the autumn immense numbers of larvæ (up to ·51 mm. in length) are found in company with one or two adults in each host. These larvæ may be divided equally into females, whose genital glands, paired and situated in the middle of the body, have only attained to a rudimentary development, and males (Text-fig. 11, A), in which the testis, situated posteriorly in the body, often contains mature spermatozoa. When in this stage the larvæ bore through the walls of the alimentary canal and disappear. No intermediates are known between these forms and the adults 3–5½ mm. in length, with a single exception to be described later. In the adult condition there is only one class of individual with a long and vastly convoluted gonad opening to the exterior in the very posterior position which is occupied by the anus, in nematodes with a functional alimentary canal. It is this circumstance which led zur Strassen to derive the adults from the male larvæ, for if they are developed from female larvæ there must have occurred a

[1] Zur Strassen, 'Zeitschr. f. wiss. Zool.,' t. 54, 1892, pp. 656–747.

shifting of the gonad during growth from a median to a posterior situation, and the conversion of a double rudiment into a single mature organ. In the most advanced male larvæ the gonad is completely occupied by a brownish mass of spermatozoa save for an apical cluster of indifferent cells (inset to Text-fig. 11, A), and zur Strassen supposes that when the larvæ begin to grow rapidly these cells proliferate and form an ovary. In a single example of ·75 mm. length (Text-fig. 11, B) the testis was represented by a receptaculum seminis full of spermatozoa, and this was succeeded by an ovary still slightly developed and only posteriorly situated. In the adult (Text-fig. 11, C) the growth in size of the gonad has been so enormous that the whole of the body-cavity is occupied by it. The ovary and oviduct together form a narrow tube running twice the length of the body. Then succeed the receptaculum seminis, and lastly, the uterus, with a diameter nearly equal to that of the animal itself, runs from near the anterior end to the genital aperture. The great difference between this and the intermediate stage has been effected by the growth of the uterus with the fertilisation of the eggs.

Though in the absence of other intermediate forms it is impossible to produce clear proof that events take their course as indicated above, yet it is probable that the female sex, though represented by larvæ, disappear without functioning, while in the males, after the spermatozoa have been formed, ova are produced in large quantities by the residual cells of the gonad. The evidence for the derivation of hermaphroditism in Rhabditis and Diplogaster from the female, and in Bradynema from the male, is in both cases of essentially the same nature, and depends on—

(1) The recognition both in the original sex and the hermaphrodite derived from it, of a constant pattern of reproductive organ.

(2) The discovery that the gonad of one sex is capable of developing the gametes of the other sex.

If zur Strassen's explanation is accepted, then in the limits of the Nematoda it is found that now the female, now the male, carries the characters of the other sex in a latent state, and when these are wakened to activity secondary hermaphroditism is developed. In Mendelian terminology either sex may be heterozygous. Moreover if the cytological phenomenon described by Maupas (p. 491) for Rhabditis elegans really shows that the male in that species is heterozygous, we are then forced to the hypothesis that both sexes are heterozygous in one and the same species, and at the same time. The phenomena of cytology and heredity as at present known in other groups, e.g. the Insecta, are capable of such diverse interpretations that it is impossible to say whether such a case as this suggested above is anomalous or no.

(5) Self-fertilisation in Animals.

Among hermaphrodite animals authentic cases of self-fertilisation are by no means common. In the Trematoda the rule of cross-fertilisation may occasionally be departed from, but only possibly in cases where the spermatozoa discharged into the body-cavity of the host find their way back into the female aperture of the same individual. Very little is known about the methods of fertilisation in the Cestoda. The evidence for self-fertilisation rests upon two observations, one by Leuckart of a penis inserted in the vagina of the same proglottis, and the other by Pagenstecher of similar relations between penis and vagina of adjacent proglottides.[1]

In the Mollusca it is easier to prove by the isolation of individuals the possibility of reproduction without cross-

[1] In the Rhabdocœl Turbellaria self-fertilisation is a very widely spread phenomenon and often the usual method of reproduction. Its existence has been put beyond doubt by the observations of individuals raised from the egg, but such experiments have not apparently been continued over several consecutive generations. In some forms the penis effects self-impregnation, in others there is no copulatory organ or female aperture and the spermatozoa migrate through the body tissue to the ovary (see Bresslau, 'Verh. deutsch. zool. Gesell.,' 1903, p. 126, and especially 'Sekera Zool. Anz.,' Bd. xxx, 1906, pp. 142–153). It must be noticed that in the three chief cases, the Turbellaria, the Nematoda,

fertilisation. A. H. Cooke quotes two cases in the Cambridge Natural History, volume "Mollusca." In both Arion ater and Linnæa auricularia, individuals isolated from birth produced fertile spawn, although in somewhat limited quantities.

In the Annelids a case has recently been described by Pierantoni[1] in Protodrilus. Ova are developed in the anterior segments, spermatozoa in the posterior, and a large proportion of the former are fertilised while still in the body-cavity. There is, however, a second method of reproduction, when by the rupture of the body-wall of the hermaphrodite the whole number of the eggs is discharged into the sea. At the same time certain male individuals commonly occurring in the species emit their spermatozoa, which unite with such eggs of the hermaphrodite as have escaped self-fertilisation.

In the Crustacea hermaphroditism is largely developed in two groups, the Isopoda and the Cirripedia. In the former, the production of the spermatozoa in each individual precedes that of the ova, and the absorption of surplus spermatozoa by phagocytes may preclude the possibility of self-fertilisation (e.g. Danalia[2]). In the cirripedes adjacent individuals normally cross-fertilise; a single case of self-fertilisation was recorded in Pollicipes (Gruvel). In the curious parasitic group, the Rhizocephala, both Sacculina and Peltogaster, invariably practise self-fertilisation.[3]

Great interest attaches to the restriction of sperm-production accompanying the condition in this group. A small part and the Rhizocephala, the self-fertilisation which they practise is evidently a secondary and adaptive phenomenon. In the first two cases it has been developed as a means by which the actual existence of the race may be safeguarded, for both classes of creatures are liable to sudden extinction by the desiccation of the pool or moist soil, where they respectively live, and it is a manifest necessity that an isolated survivor should be capable of independent reproduction when conditions again become favourable.

[1] 'Fauna u. Flora Golfes von Neapel,' t. 31, "Protodrilus," 1908, pp. 117–119.

[2] G. W. Smith, 'Fauna u. Flora Golfes Neapel,' Mon. 29, "Rhizocephala," 1906, p. 101.

[3] G. W. Smith, loc. cit., pp. 21–24.

of the testis only is used for the formation of spermatozoa, and to prevent squandering of the slender stock the maturation of the spermatozoa is completed punctually just after a brood of eggs enters the mantle-cavity.

Both the Rhizocephala and the Nematoda, the two best cases of self-fertilisation, show one advantage obtained by the animal which adopts this method of reproduction, and that is the need for a reduced number of spermatozoa. In Sacculina the economy has been effected by a special change, to be looked upon in the light of an adaptation, but in Rhabditis and Diplogaster, as we have seen, the small and markedly insufficient quantity of spermatozoa shows a recent entrance into the hermaphrodite condition, and only because every spermatozoon fertilises an egg do these forms succeed in maintaining themselves.

In the Tunicata, a group in which hermaphroditism has established itself completely, the ova ripen before the spermatozoa, and cross-fertilisation appears to be general. In Ciona ripe ova and spermatozoa are found in the ducts at the same time, and Castle[1] found that if the products from the same individual are mixed, as a rule fertilisation did not occur. This result is so significant that it is not surprising that the experiment should have been repeated. Morgan[2] found some variation in the degree of self-sterility, but generally endorsed Castle's results. In experiments which I carried out at Naples on the same tunicate in the early part of 1906 (and in which every care was taken to avoid contamination with foreign sperm), the eggs of an individual were found to be as fertile with their own spermatozoa as with those of other individuals, yielding in both cases nearly 100 per cent. of embryos. The pathological development which Castle found characteristic of self-fertilised embryos did not occur in my experiments. In conclusion, it seems possible

[1] Castle, W. E., "The Early Embryology of Ciona intestinalis," 'Bull. Mus. Comp. Zool.,' xxvii, 1896.

[2] Morgan, T. H., 'Journ. Exp. Zool.,' i, 1904, p. 137. 'Biol. Bull.,' viii, 1905.

that the American form of Ciona intestinalis differs markedly, at least in its physiology, from the Mediterranean type species, and that, as is illustrated in plants, species which differ but little from each other in external appearance may be respectively easily capable of self-fertilisation and entirely restricted to cross-fertilisation.

The free-living nematodes easily lend themselves to an investigation of the effects of continued self-fertilisation. Maupas organised cultures for this purpose, taking great care that the eight hermaphrodites chosen in each generation as the parents of the next should in no case have come into contact with mature males. With Rhabditis elegans, the period of experiment lasted from the beginning of December to the end of June, and in these seven months fifty-two consecutive generations were reared. During the whole of this time no decline in vigour or productivity could be ascribed to the continuance of self-fertilisation. It is true that immediately afterwards the race became extinct owing to the onset of sterility, but the cause of this may well be traced to a sudden rise of temperature in the month of June (Maupas, p. 493). That this is the true explanation is indicated by the fact that Rhabditis duthiersi, another hermaphrodite species, which had only been isolated from the possibility of cross-fertilisation for a few weeks, became sterile at exactly the same time when its cultures were subjected to the same conditions.

In my own researches Diplogaster maupasi has existed in cultures with no possibility of a cross through twenty-five generations, and that with not the slightest deterioration of the strain. It is hoped that under temperature conditions more equable than those of Maupas' laboratory at Algiers it will be possible to prove that self-fertilisation may continue through a longer period and larger number of generations than was the case in R. elegans.[1]

[1] The cultures have now (June 21st. 1910) been carried over forty-six generations without cross-fertilisation with no observable diminution in fertility.

Summary of Results.

In the preliminary summary on page 436, a short statement is given of Maupas' results alone. In the present paper these are completely confirmed where the material allowed, and in some of the following details the study of hermaphroditism in Rhabditis and Diplogaster has been pursued further.

(1) In one hermaphrodite species, Diplogaster maupasi, the residual males are much more numerous than in any other yet studied, and in small cultures may reach 30 per cent. of the whole number of individuals.

(2) The male secondary sexual chararacters, i.e. bursal papillæ and accessory copulatory spicule, show great variability.

(3) The production of males is cyclical, periods (each lasting a few generations) when males are frequent alternating with others in which only hermaphrodites are produced.

(4) Attempts to affect the sex-ratio artificially proved unsuccessful. It was also found impossible to increase the proportion of males by selection from favourable cultures. No rule could be discovered governing the constant fluctuations of production.

(5) Even when males were most common there was no tendency to find female or partially hermaphrodite individuals, and the males were sexually inactive. This contrasts with the conclusions reached by Maupas on Rhabditis.

(6) The number of fertile eggs laid by D. maupasi is subject to wide variation.

(7) In Rhabditis gurneyi a far greater number of fertile eggs may be produced by single individuals than in any other hermaphrodite species. The fertility is probably as great as the average bisexual species.

(8) The formation of spermatozoa is not confined to the anterior end of the gonad as in other species, but may occur in any part and at any time throughout maturity. Frequently a number of sterile eggs were laid at the onset of maturity owing to the retarded production of the spermatozoa.

(9) No males have been observed in this species, so that they are either excessively rare or extinct. R. gurneyi, then, represents a much more complete and sufficient type of hermaphroditism than has hitherto been recorded in the free-living nematodes.

(10) Self-fertilisation has formed the exclusive means of propagation throughout twenty-five[1] generations of Diplogaster maupasi without any deterioration in the character of the stock.

[1] Now forty-six. (See note on preceding page.)